The fenridazon-potassium HPLC residue analytical method is reproducible and sensitive for wheat grain and straw.

Registry No. Fenridazon-potassium, 83588-43-6.

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Lipid Class and Fatty Acid Compositions of Young *Amaranthus gangeticus* L. Leaves

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The *Amaranthus gangeticus* (Amaranthaceae) leaves yielded on extraction with chloroform–methanol 10.6% lipids (dry weight), which were separated into nonpolar lipids (53.6%), glycolipids (33.8%), and phospholipids (12.6%) by silicic acid column chromatography. These were further fractionated into subclasses by thin-layer chromatography. The nonpolar lipids were made up (wt %) of pigments (8.1), hydrocarbons (4.9), ester waxes (1.8), fatty acid methyl esters (2.7), triacylglycerols (6.4), fatty acids (5.6), diacylglycerols (5.6), sterols (9.3), monoacylglycerols (4.7), and unidentified components (4.5). The glycolipids comprised (wt %) monogalactosyl diglycerides (15.6), steryl glycosides (4.1), cerebrosides (6.8), and digalactosyl diglycerides (7.3). The phospholipids consisted (wt %) of cardiolipin (2.0), phosphatidylglycerol (3.1), phosphatidylethanolamine (3.2), phosphatidylinositol (1.7), and phosphatidylcholine (2.6). The usual fatty acids were found in varying concentrations in different lipid classes. *trans*-3-Hexadecenoic acid amounted to 12.3% in phosphatidylglycerol fatty acids.

Amaranthus gangeticus Linn. (Amaranthaceae) is a widely cultivated plant. The leaves of the young plants are used in culinary preparations all over India. There is no information on the lipids. This paper reports for the first time the nature and contents of various lipid classes as well as the constituent fatty acids present in a typical sample of young leaves normally used in culinary preparations.

EXPERIMENTAL SECTION

Extraction and Purification of the Lipids. *A. gangeticus* plants (174) were uprooted at random on the 20th day after sowing the seeds in a prepared plot. The leaves were separated. Moisture content was determined on a portion of pooled leaves. The remaining leaves were weighed and dipped in hot water to inactivate the leaf lipases (Haverkate and Van Deenen, 1965). The lipids were extracted and purified according to Folch et al. (1957). An aliquot of the chloroform fraction was used for determining the lipid content.

Separation of Nonpolar and Polar Lipid Classes. The leaf lipids were separated on a silicic acid column by using chloroform, acetone, and methanol (Carroll, 1976). The chloroform eluate contained the nonpolar lipids from which pigments were separated by passing through a charcoal–Celite column (Khor, 1979). The acetone fraction contained glycolipids and the methanol fraction phospholipids.

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Table I. Lipid Class Composition (Weight Percent) of Young *A. gangeticus* Leaves[a,b]

nonpolar lipids (53.6)	
pigments	8.1
hydrocarbons	4.9
ester waxes	1.8
fatty acid methyl esters	2.7
triacylglycerols	6.4
fatty acids	5.6
diacylglycerols	5.6
sterols	9.3
monoacylglycerols	4.7
unidentified	4.5
glycolipids (33.8)	
monogalactosyl diglycerides	15.6
steryl glycosides	4.1
cerebrosides	6.8
digalactosyl diglycerides	7.3
phospholipids (12.6)	
cardiolipin	2.0
phosphatidylglycerol	3.1
phosphatidylethanolamine	3.2
phosphatidylcholine	2.6
phosphatidylinositol	1.7

[a] Tetradecane, myristyl palmitate, methyl stearate, sesame oil, commercial monoglycerides, oleic acid, and stigmasterol were used for identification of nonpolar lipids. Authentic glycolipid and phospholipid classes, and sulfolipids that were not detected, were used as reference. [b] Overall recovery of total lipids after column chromatography followed by TLC was 92.3%.

Fractionation of Lipid Classes. Preparative thin-layer chromatography (TLC) on 0.8-mm layers of silica gel G using a solvent system of petroleum ether (40–60 °C)–diethyl ether–acetic acid (90:10:1 v/v) (Mangold and

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Table II. Fatty Acid Composition of Lipid Classes of Young *A. gangeticus* Leaves

lipid classes	fatty acid, wt %[a]									
	lauric	myristic	myristoleic	palmitic	palmitoleic	stearic	oleic	linoleic	linolenic	arachidic
nonpolar lipids										
ester waxes	5.8	4.2	0.9	28.8	1.5	13.0	9.9	7.6	27.4	0.9
fatty acid methyl esters	11.1	2.9	0.7	22.2	1.5	9.0	7.8	3.1	41.3	0.4
triacylglycerols	1.6	0.9	0.6	26.0	11.7	4.7	3.5	7.4	43.2	0.4
fatty acids	4.9	4.4	0.5	28.9	1.9	13.4	9.5	7.9	27.5	1.1
diacylglycerols	1.4	1.3	0.1	18.9	0.4	8.4	22.8	33.8	12.2	0.7
monoacylglycerols	3.5	4.6	0.5	28.8	1.3	10.0	16.6	21.2	9.9	3.6
glycolipids										
monogalactosyl diglycerides	0.9	0.3	ND	2.3	0.2	0.6	0.7	1.5	93.4	T[d]
cerebrosides	6.7	1.0	ND	19.8	ND	11.1	7.6	2.4	51.4	ND
digalactosyl diglycerides	0.5	ND[c]	ND	16.2	0.2	3.5	1.7	2.7	75.2	ND
phospholipids										
cardiolipin	2.4	1.1	0.3	15.7	9.9	1.9	3.2	14.2	51.2	0.1
phosphatidylglycerol	1.7	1.0	0.6	23.1	12.3[b]	4.7	3.6	8.0	44.8	0.2
phosphatidylethanolamine	3.7	2.5	1.0	29.9	7.0	12.3	7.0	12.2	23.7	0.7
phosphatidylinositol	4.9	4.5	0.7	36.0	0.6	14.8	9.8	15.2	13.2	0.3
phosphatidylcholine	2.8	1.8	0.5	38.6	1.7	9.1	7.6	23.0	14.9	ND

[a] Duplicate values for methyl esters in standard mixtures by GLC analysis varied within 10% for minor components (<5%) and within 3% for others. [b] *trans*-3-Hexadecenoic acid. [c] ND = not detected. [d] T = trace (<0.1%).

Malins, 1960) separated the nonpolar lipids into various subclasses (Table I). Di- and monoacylglycerols that were not resolved in this system were separated by using petroleum ether–diethyl ether–acetic acid (60:40:1 v/v). Each lipid class was estimated by gravimetry. The glycolipid classes were separated on silica gel G by using chloroform–methanol–acetic acid–water (170:24:25:4 v/v) (Nichols, 1970) and quantified by estimation of their carbohydrate moiety using the anthrone reagent (Yamamoto and Rouser, 1970). The phospholipid classes were separated by TLC on silica gel G using chloroform–acetone–methanol–acetic acid–water (60:80:20:20:10 v/v) (Rouser et al., 1970) and quantified by spectrophotometry after color development (Harris and Popat, 1954). Lipid classes were identified by using authentic samples (footnote *a*, Table I) as reference compounds, rerunning with different solvent systems and spraying with the following group specific spray reagents (Kates, 1972): concentrated sulfuric acid–acetic acid (1:1 v/v) for sterols, Dragendorff reagent for choline group, ninhydrin for free amino group, periodate–Schiff reagent for vicinal hydroxyl group, α-naphthol for sugar, and ammonium molybdate–perchloric acid for phosphate-containing lipids.

Gas–Liquid Chromatography (GLC) of Fatty Acid Methyl Esters. Acyl lipids, except free fatty acids and ester waxes, were transesterified with methanol containing 1% sodium methoxide (Luddy et al., 1960). Free fatty acids were converted to methyl esters with diazomethane–diethyl ether in methanol (Luddy et al., 1960). Ester waxes were saponified and the liberated fatty acids were esterified as in the case of free fatty acids. Methyl esters were purified by preparative TLC on silica gel G using petroleum ether–diethyl ether–acetic acid (90:10:1 v/v). Methyl esters of each lipid class were qualitatively analyzed by TLC on silica gel G impregnated with 9% silver nitrate using petroleum ether–diethyl ether (90:10 v/v). The methyl esters of phosphatidylglycerols contained *trans*-monoene, which was analyzed by GLC for chain length. The double-bond position was determined by permanganate–periodate oxidation followed by GLC analysis after esterification of the acids with diazomethane (Christie, 1973). Methyl esters were analyzed by using a Hewlett-Packard 5840 A gas chromatograph fitted with a flame ionization detector and a data processor. A glass column (1.8 m × 4 mm i.d.) packed with 10% DEGS on Chromosorb-W HP was used. The column, detector, and injection port were maintained at 190, 300, and 250 °C, respectively. The nitrogen flow rate was 30 mL/min. The peaks were identified by comparison with standard fatty acid methyl esters.

RESULTS AND DISCUSSION

Lipid Class Composition. The leaves contained 88.0% moisture and 1.27% of total lipids amounting to 10.6% on a dry weight basis. The lipids were quantitatively fractionated into nonpolar lipids, glycolipids, and phospholipids and their subclasses by column chromatography and TLC. The lipid class composition is shown in Table I. About half the quantity of total lipids was accounted by nonpolar lipids, one-third by glycolipids, and the rest by phospholipids. Glycopids and phospholipids of leaves, in general (Hitchcock and Nichols, 1971; Mudd and Garcia, 1975), have been well investigated but not the nonpolar lipids. The nonpolar lipids of leaves of young *A. gangeticus* plants consisted of pigments, hydrocarbons, ester waxes, fatty acid methyl esters, triacylglycerols, fatty acids, diacylglycerols, sterols, and monoacylglycerols (Table I). The pigments and sterols were the major fractions. Hydrocarbons and ester waxes were perhaps present on the leaf surface for biological functions (Kolattukudy, 1975). The contents of acyglycerols were not as high as observed in other leaf lipids (Weenink, 1961). The fatty acid methyl esters were perhaps artifacts formed during extraction with chloroform–methanol as suggested by Lough et al. (1962). The glycolipids comprised monogalactosyl diglycerides (MGDG), which predominated others, steryl glycosides, cerebrosides, and digalactosyl diglycerides (DGDG). With the exception of lucerne (alfalfa), other leaves studied contained more MGDG than DGDG (O'Brien and Benzon, 1964; Hawke, 1973). The phospholipids included cardiolipin, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylcholine, which are normally found in other plant leaves. Phosphatidylserine was not found as in some other plant leaves (Kates and Marshall, 1975). Sulfolipids were also not detected.

Fatty Acid Composition. The major fatty acids of leaves in general were linolenic, linoleic, and palmitic (Hitchcock and Nichols, 1971); *A. gangeticus* leaf lipids were no exception (Table II). These were found in varying proportions among different lipid classes. The predominant acid was linolenic in methyl esters and triacylglycerols, linoleic in diacylglycerols, and palmitic in monoacylglycerols, fatty acids, and ester waxes. All three classes of glycolipids contained linolenic acid in predom-

inant proportion, as in other leaf glycolipids (Mudd and Garcia, 1975). The chief fatty acid was linolenic in cardiolipin and phosphatidylglycerol and palmitic in the other phospholipid classes. Significant proportions of lauric acid were also found in many of the lipid classes. Triacylglycerols, phosphatidylglycerol, cardiolipin, and phosphatidylethanolamine contained appreciable proportions of palmitoleic acid. The occurrence of this acid in a number of leaves is known (Hitchcock and Nichols, 1971). Phosphatidylglycerol contained *trans*-3-hexadecenoic acid. This acid is frequently present in phosphatidylglycerols of photosynthetic tissues (Hitchcock and Nichols, 1971).

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Registry No. Lauric acid, 143-07-7; myristic acid, 544-63-8; myristoleic acid, 544-64-9; palmitic acid, 57-10-3; palmitoleic acid, 373-49-9; stearic acid, 57-11-4; oleic acid, 112-80-1; linoleic acid, 60-33-3; linolenic acid, 463-40-1; arachidic acid, 506-30-9; *trans*-3-hexadecenoic acid, 1686-10-8.

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Determination of Volatile Fatty Acids in Molasses by Gas–Liquid Chromatography of Their Benzyl Esters

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The determination of formic, acetic, propionic, and *n*-butyric acids in cane molasses by gas–liquid chromatography is described. The acids were isolated by selective elution from silicic acid, using dichloromethane/ethanol (95/5). The benzyl esters, prepared by reaction of the tetrabutylammonium salts of the acids with benzyl bromide in acetone, were analyzed on a column of 20% DEGS on Chromosorb W by using benzyl *n*-valerate as the internal standard. Recovery of these acids (0.25–4 mg) from molasses averaged 99.7 ± 3.6%. The method applied to the analysis of a range of commercial molasses samples showed that formic (1.1–4.1 mg/g) and acetic (3.1–3.7 mg/g) acids were predominant, with traces (<0.1 mg/g) of propionic and *n*-butyric acids.

Molasses is widely used as a growth medium in the fermentation industry, due to its high content of fermentable sugars. Typically these include sucrose (30–40%), glucose (4–9%), and fructose (5–12%) (Paturau, 1982; Baker, 1980). In addition, the molasses contributes significant amounts of other nutrients, such as amino acids, minerals, and vitamins (Paturau, 1982; Baker, 1980; Schiweck, 1980). However, it may also contain small amounts of volatile carboxylic acids, which have been found to have an inhibitory action in fermentation (Labendzinski, 1980; Baker, 1980; Dierssen et al., 1956). It has been previously shown that these can occur in molasses at concentrations up to 0.4% (formic acid), 1% (acetic acid), 0.3% (propionic acid), and 0.6% (*n*-butyric acid) (Dierssen et al., 1956; Baker, 1980). Since the inhibitory level of these materials may be quite low (about 0.2% for formic acid) (Schiweck and Harberl, 1973), a convenient method for their estimation is required.

Previously reported methods for estimation of carboxylic acids in molasses have included paper chromatography (Dierssen et al., 1956), solvent extraction followed by either

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